-pH suspected GHB/GBL sample as soon as possible to understand GHB/GBL interconversion rates and which species of GHB exist in solution.

The Importance of pH
Forensic Issues of Interconversion

- **Deionized water at ambient temperature-GHB stable**
  - **GBL converts to GHB: 1% GHB after 1 day, < 3% after 16 days, 16% after 2 months and 33 % after 6-7 months**
- **pH 12- GHB stable and GBL rapidly converts to GHB**
- **pH 7- Conversion of GBL to GHB similar to base catalyzed mechanism**
- **pH 2- Start with GHB or GBL-mixture of both forms within 9 days**

*Source:L. Ciolino and M. Mesmer FDA Forensic Chemistry Center, February 2000 Academy Meeting Presentation
(*Consideration for legal reclassification: Any Aqueous GBL solution has the potential to contain GHB)
At pH 1, GHB starts converting to GBL.

Step #1-To Screen for All Drugs
-Filter or centrifuge samples that have an excessive amount of insoluble particulates.
-Add 2-3 drop of liquid sample in a 1.8 mL vial and bring up to 1 mL with water. For very viscous samples, adjust volumes and dilute appropriately. For weak unknowns, samples may not need any diluting. Screen on a GC instrument to normalize GHB/GBL/1,4-BD to approximately 1-3 mg/mL. **This solution will ideally become a stock solution for Steps 2-4. If concentrations of GHB/GBL/1,4-BD are vastly different, individual stock solutions may need to be prepared for steps 2-4. If an ample amount of stock solution exists, use fresh stock solution when step #4 is performed instead of using the water fractions left over from step #2. Any remaining GHB could re-establish a new equilibrium with GBL and reduce GHB concentrations for derivatization. If the unknown contains a lot of 1,4-BD, one can use stock solution for step 4 or the water fraction remaining from step #3.** (If an unknown contains insoluble matter of forensic interest, prepare a second vial with the solid matter dissolved in methanol and 2 drops of chloroform if necessary.) Submit unknowns to GC/MS for **screen** testing using the GHBSCRN method. This will screen for GHB/GBL/1,4-BD and for other solubilized drugs, although the volatility of some drugs will likely be reduced in aqueous solutions. Confirmatory work is described below.

Step #2-To Confirm GBL in Aqueous Liquids
-Extract aqueous samples from **Step #1** with an equal volume of CHCL3 to isolate GBL and to remove extraneous organic components. Isolate the chloroform layer and wash 3-5X with water to remove any GHB free acid. The GHB free acid partition coefficient for chloroform is very small but it does exist, being optimal at pH 2 and saturated with NaCl. The absence of GHB for this extraction procedure should be validated for an acidic solution (pH 2-3) by evaporating the water washed chloroform layer and derivatizing the residue. The chloroform layer will not extract GHB in the **anionic** state whether GHB came from a sodium salts, another salt form, or from free acid.
-Dry chloroform layer with anhydrous sodium sulfate. (Optional)
-Run the chloroform extract on the GC instrument with a 1mg/mL GBL standard prepared in chloroform. Use area counts to dilute unknowns to approximately 1 mg/mL GBL for GC/MS confirmation.
-Chromatographic fronting/splitting exists for GBL in water on the GC/MS instrument. A GBL standard prepared in chloroform volatilizes better, does not front/split and is better suited for routine confirmatory work requiring less injector maintenance.

Step #3-To Confirm 1,4-Butanediol (1,4-BD) in Aqueous Liquids

-The NY DEA and New Hampshire State Police Forensic Laboratory have protocols that imply 1,4-butanediol can be extracted from aqueous liquids with chloroform. I was not able to validate this extraction procedure with any degree of success. However, the above procedure can be used to confirm 1,4-BD if chloroform is substituted with Petroleum Ether.

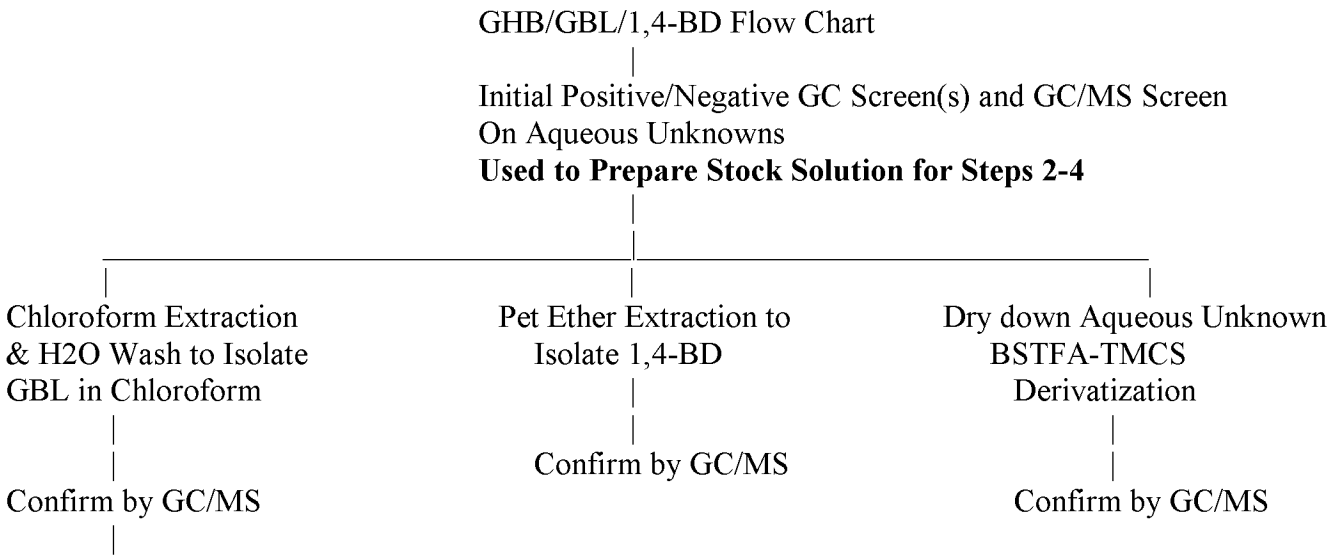
Step #4- To Confirm GHB in Aqueous Solutions

-If appropriate, place 0.5 mL of diluted sample (from **step #1**) in a 1.8 mL vial (amber and calibrated) and extract with chloroform-Optional- This step may removes extraneous organic components that might compete for BSTFA-TMCS and it also removes GBL even though evaporating the sample will also remove GBL.) Evaporate at room temperature using the Pierce Reacti-Therm unit. Two days may be required to thoroughly evaporate the water. This process will remove GBL and 1,4-butanediol along with water by evaporation. Only GHB can be confirmed if present in the sample. Derivatize **immediately** after evaporating.

-While drying unknowns, remove 0.5 mL of a stock 1 mg/mL aqueous GHB standard solution for evaporation as well. GHB is stable in de-ionized water and any minor interconversion (if any) will be evaporated away.

-Once the sample (standard) is dried down, add 200ul of BSTFA-TMCS. Let sit at room temperature for 2-3 hours, mixing/vortex repeatedly. "BSTFA-TMCS has good solvent properties and can function as a silylation reagent without additional solvents." *Supelco Certificate of Analysis

-After derivatization, add an appropriate volume of acetonitrile to bring the final volume back to 0.5 mL. Confirm using GC/MS with a derivatized GHB standard within 24-48 hours. Submit a blank consisting of BSTFA-TMCS and acetonitrile to rule out GHB contamination of the solvents and instrument contamination.



If an unknown is mostly GBL, adjust stock solution or use undiluted unknown for the BSTFA-TMCS step. GHB will be present in aqueous GBL solution of any pH.

Reference

* The Extraction and Infrared Identification of Gamma Hydroxybutyric Acid (GHB) from Aqueous Solutions, Chappell, Meyn, and Ngim, 2004.